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Genetic polymorphisms of the renin-angiotensin-aldosterone system in end-stage renal disease

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Genetic polymorphisms of the renin-angiotensin-aldosterone system in end-stage renal disease.

Background. Hypertension contributes to the progression to renal failure. A genetic susceptibility to hypertension may predispose to the development of end-stage renal disease (ESRD) and promote a more rapid progression to ESRD in patients with renal diseases. Genes encoding for angiotensinogen (*AGT*), angiotensin-converting enzyme (*ACE*), and aldosterone synthase (*CYP11B2*) are candidates for abnormal blood pressure regulation.

Methods. Genotyping was performed in 327 control subjects and 260 ESRD patients for the M235T-*AGT*, the insertion/deletion (I/D)-*ACE*, and the −344T/C-*CYP11B2* gene polymorphisms using polymerase chain reaction, gel analysis, and appropriate restriction digest when required.

Results. Genotype frequencies did not differ significantly between ESRD patients and controls. When ESRD diabetic subjects were compared with diabetic patients without nephropathy, the prevalence of the *AGT-MM* genotype was lower (28.1 vs. 52.8%, $P < 0.01$), while the *AGT-TT* genotype was higher (15.6 vs. 2.7%, $P < 0.05$). The *AGT-TT* genotype was associated with a faster progression to ESRD in patients with glomerulonephritis ($P < 0.05$). In the total ESRD population, progression of renal disease was faster with the *ACE-DD* than with the *DI* and *II* alleles ($P < 0.05$). This association was particularly strong when the interaction with the *AGT* genotype was analyzed, with a rapid progression in *ACE-DD* as compared with *ACE-DI* and *II* in patients with the *AGT-MM* genotype ($P < 0.01$).

Conclusions. Susceptibility for ESRD and faster progression to ESRD are linked with the *AGT* genotype in diabetic patients. Faster progression to ESRD is associated with the *ACE* genotype when the total population with ESRD and with the *AGT* genotype when patients with glomerulonephritis are considered. Thus, genes of the renin-angiotensin-aldosterone system are candidate genes for further understanding of the inter-individual differences in the development and course of ESRD.

Key words: angiotensinogen, hypertension, aldosterone synthase, renal failure, blood pressure, progressive renal disease.

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Nephropathies of any etiology tend to progress to end-stage renal disease (ESRD) more or less rapidly over time [1]. The resulting costs of treatment for ESRD are enormous. Hypertension is the major contributor in the progression of renal failure in patients with renal disease both with and without proteinuria [2]. Moreover, hypertension per se is a risk factor for the development of ESRD [3, 4]. On the other hand, the prevalence of hypertension increases with decreasing renal function [5]. This results from both a decreased sodium excretion and an activation of the renin-angiotensin-aldosterone system (RAS). Therefore, it is possible that a genetic predisposition to salt-dependent hypertension or overactivation of the RAS may (*1*) predispose to the development of renal failure and (*2*) promote a more rapid loss of glomerular filtration rate in patients suffering from renal diseases. Thus, genes that regulate renal sodium reabsorption or genes of the RAS may be extremely important in patients suffering from ESRD. Among the candidate genes of the RAS, the angiotensinogen gene (*AGT*), the angiotensin-converting enzyme gene (*ACE*) and the aldosterone synthase gene (*CYP11B2*) are of particular interest.

Two molecular variants of the *AGT*, the *T174M* and *M235T*, were analyzed in an associated study in selected hypertensive subjects in two distinct American and French populations [6]. Both *T174M* and *M235T* variants were significantly more frequent in all hypertensive cases than in controls, with a further increase in frequency among the more severely affected cases. Moreover, the *M235T* variant was associated with a high plasma level of *AGT* in hypertensive subjects.

An insertion/deletion (I/D) polymorphism in intron 16 of the *ACE* has been described by Rigat et al [7]. In subjects with the deletion polymorphism (*DD*), plasma ACE activity was fourfold higher than in subjects homozygous for the insertion allele, suggesting that the *DD* polymorphism could be a marker for a high serum ACE level intermediate phenotype [7]. The *DD* polymorphism has been associated with an increased risk of myocardial infarction [8], although not with the development of coronary stenosis [9] and with idiopathic dilated cardiomy-

Table 1. Baseline demographic characteristics of the 587 subjects studied, subdivided by population groups

	Control subjects ($N = 327$)			ESRD patients ($N = 260$)	
	Healthy	Hypertension	Diabetes	Dialysis	Transplantation
N	162	128	37	102	158
Sex *Male/Female*	95/67	70/58	21/16	59/44	89/69
Age *years*	49 ± 16	59 ± 17[b]	52 ± 21	64 ± 13[b]	48 ± 12[c]
BMI kg/m^2	23.5 ± 4.0	28.1 ± 6.0[b]	25.9 ± 4.6[a]	25.0 ± 4.3[a]	24.9 ± 4.6[a]
Blood pressure *mm Hg*					
Systolic	129 ± 15	154 ± 21[b]	144 ± 23[b]	150 ± 26[b]	137 ± 14[bc]
Diastolic	78 ± 9	90 ± 14[b]	82 ± 9[a]	81 ± 15	86 ± 8[bc]

Abbreviations are: ESRD, end-stage renal disease; BMI, body mass index.
Data are mean ± SD.
[a] $P < 0.01$, [b] $P < 0.0001$ vs. healthy controls
[c] $P < 0.0001$ vs. dialysis patients

opathy [10]. In a small group of patients with essential hypertension, the percentage of *DD* polymorphism was higher in hypertensive subjects whereas the II polymorphism was more frequent in normotensive controls [11]. Homozygosity for D allele was associated with an increased risk of developing ESRD at early age in polycystic kidney disease patients [12, 13] and with a shorter time of graft survival in kidney transplant recipients [14]. However, in IgA glomerulonephritis (IgA GN), genotype distributions and allele frequencies were not significantly different between controls and patients with nephritis and stable renal function [15].

Expression of the *CYP11B2* gene is regulated by angiotensin II through cAMP-dependent modulation of the gene promoter region containing a variety of control factors, one of which is the steroidogenic factor-1 (SF1) [16, 17]. Evidence for a genetic variant in the SF1 site of the *CYP11B2* was found in two independent hypertensive populations, suggesting that mutations of this enzyme may be relevant in hypertension [18, 19]. This variant consists in a single nucleotide polymorphism at position −344 of the *CYP11B2* promoter (−344C/T). Differences in the structure of this site alter the sensitivity to angiotensin II [19]. Besides hypertension, this polymorphism in the SF1 has been associated with increased left ventricular size and impaired diastolic function [20]. This finding was confirmed in another study in which the −344C variant was also associated with a higher ratio of aldosterone-to-plasma renin activity as well as with hypertension [21].

To date, no study has analyzed the prevalence and distribution, alone or combined, of the *M235T-AGT*, *I/D-ACE*, or *−344C/T-CYP11B2* polymorphisms and their relationship with the time of onset of ESRD, time from diagnosis to ESRD, and age of patients at the time of ESRD in a population of patients with chronic renal failure of comparable ethnical background but with renal diseases of different etiology. Therefore, this study aimed at establishing first whether polymorphic variants in these genes are more prevalent in ESRD patients, and second whether polymorphisms of these genes are associated with a more rapid progression to ESRD.

METHODS

Subjects

Study subjects were 260 white patients with ESRD (either dialysis or transplanted) from our Division of Nephrology and Hypertension (University Hospital of Berne, Berne, Switzerland) and 327 white control subjects without renal diseases (Table 1). The geographic origin of 519 out of 587 subjects was the Swiss German area, while the remaining 68 consisted of subjects from the North Mediterranean area ($N = 48$), Eastern Europe ($N = 14$), and Asia or Africa ($N = 6$). Basic demographic data, including current blood pressure, information on associated diseases and current medication, including the type and number of antihypertensive drugs, were obtained for all subjects. In the control group, serum creatinine was <104 μmol/L, proteinuria was <30 mg/L, and urinary sediment was normal in all subjects. All diabetic patients in the control and ESRD groups had insulin-dependent diabetes mellitus. For ESRD patients, the date of diagnosis of the renal disease, renal histology, time of onset of ESRD, type of renal replacement treatment, age at transplantation, blood pressure in the pre-ESRD period, and other information were also available. Subjects admitted to our service with serum creatinine >150 μmol/L (1.7 mg/dL) and whose renal history could not be retrieved were excluded. The time from diagnosis of the renal disease to the onset of ESRD was used as a measure for progression. A subgroup analysis by diagnosis of renal disease was performed for the following etiologies: glomerulonephritis ($N = 76$), interstitial nephritis ($N = 40$), diabetes mellitus ($N = 32$), autosomal-dominant polycystic kidney disease ($N = 30$), pyelonephritis/vesicoureteral ($N = 25$), nephroangiosclerosis ($N = 14$), and others ($N = 40$). In patients with autosomal-dominant polycystic kidney disease, progression was defined

Table 2. Oligonucleotides for amplification and screening

Gene	Sequence	Cycling conditions
AGT		
Sense	5′-CCGTTTGTGCAGGGCCTGGCTCTCT-3′	30 × (94°C 30 sec, 68°C 60 sec, 72°C 30 sec)
Antisense	5′-CAGGGTGCTGTCCACACTGGACCCC-3′	
ACE-1		
Sense	5′-CTGGAGACCACTCCCATCCTTTCT-3′	28 × (94°C 60 sec, 62°C 45 sec, 72°C 60 sec)
Antisense	5′-GATGTGGCCATCACATTCGTCAGAT-3′	
ACE-2		
Sense	5′-TGGGACCACAGCGCCCGCCACTAC-3′	28 × (94°C 30 sec, 67°C 45 sec, 72°C 120 sec)
Antisense	5′-TCGCCAGCCCTCCCATGCCCATAA-3′	
CYP11B2		
Sense	5′-CAGGGGGTACGTGGACATTT-3′	35 × (94°C 30 sec, 52°C 30 sec, 72°C 30 sec)
Antisense	5′-CAGGGCTGAGAGGAGTAAAA-3′	

Cycling conditions are number of cycles × (temperature and time of denaturation, annealing, and elongation).

as the time when serum creatinine increased >120 μmol/L until the time of onset of ESRD.

Genetic analysis for the M235T-AGT, D/I-ACE, and −344C/T-CYP11B2 genotypes was performed in all 587 subjects, and the prevalence of identified polymorphisms was analyzed in patients with ESRD as compared with control subjects for susceptibility to renal diseases and within the ESRD population for the progression of renal function loss.

DNA preparation and polymerase chain reaction analysis

Genomic DNA was isolated from peripheral blood using the Nucleon BACC3 DNA extraction kit (Amersham Intl., Buckinghamshire, UK). Detection of polymorphisms was performed by polymerase chain reaction (PCR) analysis using the primers reported in Table 2. All reactions were performed with 10 pmol of each primer in a final volume of 50 μL, containing 1.5 mmol/L $MgCl_2$ (except for ACE1 3 mmol/L $MgCl_2$), 10 mmol/L Tris HCl (pH 8.3), 50 mmol/L KCl, 200 μmol/L dNTPs, and 1 U of *AmpliTaq Gold* polymerase (PE Biosystems, Foster City, CA, USA).

Polymorphism detection for the *AGT* gene was performed by restriction typing of PCR products using the primers and the cycling conditions reported in Table 2. To introduce the second part of the half-site produced by the T→C transition at nucleotide 704 in exon 2 of the *AGT* gene, an antisense PCR primer with two mismatches was used (Table 2, primer 2, underlined).

The amplification of intron 16 in the *ACE* gene containing the insertion was performed using the primers and cycling conditions shown in the Table 2. Because the D allele in heterozygous samples is preferentially amplified, each sample found to have the DD genotype was subjected to a second independent PCR amplification with primers that recognize an insertion-specific sequence (primers $N = 5$ to 6). The reaction yields a PCR product only in the presence of an I allele and no product in samples homozygous for DD.

Polymorphism analysis of the *CYP11B2* gene was performed by restriction typing of PCR products from the promoter region of the *CYP11B2* gene using the primers and the cycling conditions reported in Table 2.

Gel analysis and genotyping

Polymerase chain reaction products were analyzed on 12% acrylamide gels containing 7.25% glycerol using a two-buffer system, either native or after appropriate restriction digest. Four microliters of the PCR sample were loaded, and DNA was visualized by silver staining [22].

For the analysis of the *AGT M235T* polymorphism, PCR products were digested with AspI for two hours at 37°C, obtaining for the *M235* variant a 165 bp fragment and a 144 bp fragment for the T235 variant.

For the analysis of the *ACE D/I* polymorphism, the PCR product shows a 190 bp fragment in the absence of the insertion (D allele) and a 490 bp fragment in the presence of the insertion (I allele). Products in which only the D fragment was present were further analyzed as described previously in this article. Where mistyping was present, a 335 bp product was identified (*ID* genotype), while no band was detected when the *DD* typing was correct.

For the analysis of the *CYP11B2-344C/T* polymorphism, PCR products were digested with HaeIII for two hours at 37°C. The *−344T* allele lacks the HaeIII site (GGCC) present in the *−344C* allele. In the presence of the *−344C* allele, the PCR product (152 bp) was cut into two fragments of 97 bp and 56 bp.

Statistics

Values are expressed as mean ± SD or percentage. Statistical differences between means were assessed by *t* test or analysis of variance (ANOVA) for analysis of continuous variables and by nonparametric analysis using the Wilcoxon or Kruskal–Wallis test for variables that were not normally distributed. For categorical variables, the 2 × 2 contingency table χ^2 test was used. The expected "disease" frequency for the target population

Table 3. Comparison of allelic frequencies in percentage among controls and ESRD patients

		AGT T	*ACE* D	*CYP11B2* T
Cases	*N*		%	
Controls	654	39.6	48.3	53.2
Normal	324	39.8	49.1	53.4
Hypertension	256	43.0	49.2	51.6
DM	74	27.0[a]	41.9	58.1
ESRD	520	42.5	45.6	51.2
Dialysis	204	42.7	42.2	51.0
Transplanted	316	42.4	47.8	51.3

[a] $P < 0.01$ vs. normal controls and patients with hypertension (ANOVA, *F* ratio 4.902).

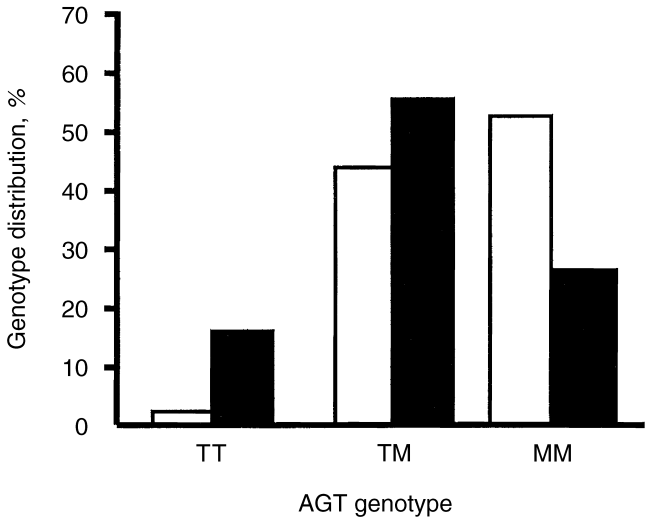

Fig. 1. AGT genotypes in patients with diabetes mellitus without nephropathy (□, *N* = 37) or with diabetic ESRD (■, *N* = 32). Cochran's linear trend $P < 0.01$.

was calculated according to the Hardy–Weinberg equation [23]. All statistical analyses were performed using the Systat 8.0 (SPSS Inc., Chicago, IL, USA) statistical software package. Polymorphic biallelic pairs are shown for the combinations supposed to protect against or predispose to high blood pressure only.

RESULTS

Baseline demographic data of the subjects investigated in the present study are outlined in Table 1 and are subdivided by the different population subgroups. Allele frequencies in controls and ESRD patients stratified by subgroups for the *AGT*, *ACE*, and *CYP11B2* genes are reported in Table 3. In the ESRD group at the time of renal diagnosis, patients were comparable for renal function and incidence of proteinuria or diabetes among the different genotype subgroups. When renal function at the time of diagnosis was analyzed for the genotype subgroups, serum creatinine was 139 ± 6 (*MM*), 136 ± 4 (*MT*), 142 ± 5 (*TT*) μmol/L when patients were subdivided for their *AGT* polymorphism; 136 ± 6 (*II*), 139 ± 4 (*DI*), and 135 ± 7 (*DD*) μmol/L when analyzed for the *ACE* polymorphism; and 141 ± 7 (*CC*), 136 ± 4 (*CT*), and 138 ± 6 (*TT*) μmol/L when the *CYP11B2* polymorphism was considered. The incidence of diabetes mellitus ranged between 10 and 17%, and the prevalence of proteinuria >1.5 g/day at diagnosis ranged between 33 and 44% among the different genotype subgroups without significant within-group differences. The genotype of the three genes studied was analyzed for an association with the susceptibility to renal diseases. The expected frequencies of the *AGT*, *ACE*, and *CYP11B2* genotypes, under the assumption of the Hardy–Weinberg equilibrium, did not differ from observed frequencies in ESRD patients and control subjects. The *AGT-235T* variant was lower in patients with diabetes mellitus than in healthy controls or controls with hypertension (*F* ratio 4.902, $P < 0.01$). Since diabetic patients without nephropathy had a lower prevalence of the *AGT-TT* alleles, the genotype and its interactions with the *ACE* and *CYP11B2* genotypes were analyzed in all 69 patients with diabetes mellitus. Observed frequencies for the *AGT* genotype in control patients with diabetes mellitus were in Hardy–Weinberg disequilibrium when compared with the expected frequencies calculated from allelic distribution in the entire diabetic population. The prevalence of the *AGT-MM* genotype was significantly higher in diabetic controls and the *AGT-TT* genotype substantially higher in diabetic ESRD patients (Pearson χ^2 $P < 0.05$, Cochran's linear trend, $P < 0.01$; Fig. 1). A comparison of biallelic frequencies alone or combined in controls and ESRD patients is shown in Table 4. Biallelic pairs are shown for the combinations that are supposed to protect against or predispose to high blood pressure as described previously in this article. Since the combined prevalence of homozygosity for these polymorphisms was low, data for the frequencies in the subgroups (healthy subjects, patients with hypertension, diabetes mellitus, on dialysis or with a transplant) are not shown.

Genetic polymorphisms were analyzed for their relationship to the rate of progression of renal disease and to blood pressure or the presence or absence of hypertension. Progression of renal disease in the ESRD population as a whole was influenced by the *ACE* polymorphism. The time from diagnosis to the onset of ESRD was shorter in the presence of the *ACE-DD* ($N = 42$) than with *DI* ($N = 155$) or *II* ($N = 63$) alleles (Kruskal–Wallis test = 8.11, $P < 0.05$; Fig. 2A). Differences between women and men in relationship to the *ACE* gene polymorphism have been suggested [24]. Progression to ESRD was more rapid in men than in women (9.5 ± 0.7 vs. 11.9 ± 1.0 years, $P = 0.042$). The presence of the *II* or

Table 4. Comparison of biallelic frequencies alone and combined among controls and end-stage renal disease (ESRD) patients

	Controls	ESRD
	%	
AGT		
MM	36.8	30.8
TT	16.4	15.8
ACE		
II	21.1	24.3
DD	19.2	15.8
CYP11B2		
CC	23.8	22.8
TT	31.6	25.5
AGT/ACE		
MM/II	7.7	8.1
TT/DD	3.4	1.5
AGT/CYP11B2		
MM/CC	8.6	6.5
TT/TT	6.1	3.1
ACE/CYP11B2		
II/CC	5.2	7.3
DD/TT	4.9	4.6

Biallelic pairs are shown for the combinations that are supposed to protect against or predispose to high blood pressure. For instance, since the AGT-M235T allele has been associated with high angiotensin levels and the ACE-DD variant was found to have higher plasma ACE activity, a combination of both (AGT-TT + ACE-DD) would more likely be associated with hypertension and ESRD than the mirror combination (AGT-MM + ACE-II).

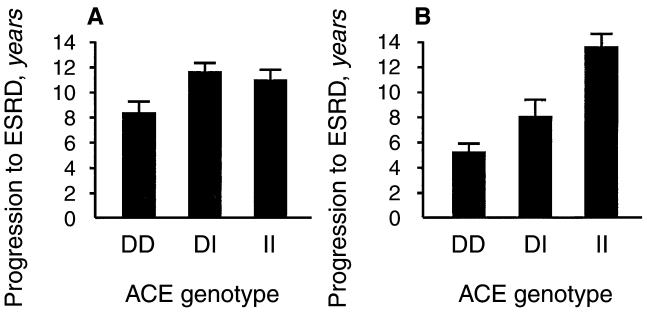

Fig. 2. (*A*) ACE genotype and rate of progression of renal disease in years from the time of diagnosis to the onset of end-stage renal disease (ESRD) in the entire ESRD population (DD vs. DI + II Kruskal–Wallis test = 8.11, $P < 0.05$). (*B*) Interaction between the AGT and the ACE gene polymorphisms on the rate of loss of renal function for 78 ESRD patients homozygous for the *AGT MM* gene (F ratio = 4.856, $P < 0.01$).

DI genotype accounted for this difference with a shorter time from diagnosis to ESRD in men than women (9.7 ± vs. 12.5 ± 1.1 years, $P = 0.036$), while the effect of the *DD* genotype on the progression to ESRD was comparable between genders (7.6 ± 1.4 vs. 9.1 ± 2.5 years). The association of progression of renal failure with the *ACE I/D* polymorphism was particularly strong when the interaction with the *AGT* genotype was analyzed, with a rapid progression in *ACE-DD* ($N = 12$) as compared with *ACE-DI* ($N = 46$) and II ($N = 20$) in patients with the *AGT-MM* genotype (F ratio = 4.856, $P < 0.01$; Fig. 2B). No significant association was found between the *AGT* or *CYP11B2* genotype and progression of renal failure

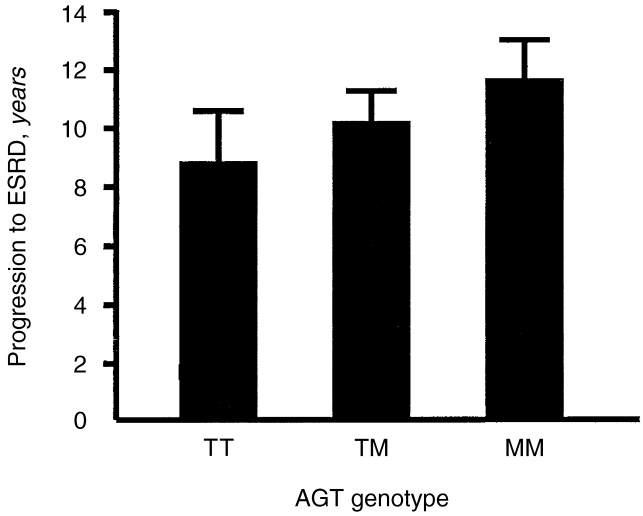

Fig. 3. AGT genotype and rate of progression of renal disease in years from the time of diagnosis to the onset of ESRD in 76 patients with glomerulonephritis (F ratio = 3.821, $P < 0.05$).

among all ESRD patients. When progression in ESRD patients was tested for an association with the underlying renal disease, the *AGT-TT* genotype was associated with a slightly higher progression to ESRD in patients with glomerulonephritis (F ratio = 3.821, $P < 0.05$; Fig. 3).

In the non-ESRD population, no relationship between either systolic or diastolic blood pressure and the *AGT*, *ACE*, or *CYP11B2* genotypes was found. The prevalence of *AGT-TT/ACE-DD* (5.5 vs. 2.4%, $P < 0.01$) was higher, and that of the *AGT-MM/ACE-II* was lower (4.7 vs. 8.6%, $P < 0.05$) in patients with hypertension than in healthy controls. In the control group, homozygosity for the *AGT-235T* genotype was associated with higher diastolic but not systolic blood pressure (144/87 ± 25/16 mm Hg, $N = 52$) as compared with the *MM* (139/81 ± 21/11 mm Hg, $N = 116$) or the *MT* (141/84 ± 21/12 mm Hg, $N = 148$) genotypes. No association was found with either the *ACE* or *CYP11B2* polymorphism and diastolic or systolic blood pressure. A general linear model analysis of the effect of the three gene polymorphisms on systolic or diastolic blood pressure did not reveal any context dependency among these gene polymorphisms and blood pressure.

DISCUSSION

We have analyzed three gene polymorphisms of the RAS for association with ESRD. The RAS is a logical target because (*1*) an increased activity of the RAS causes angiotensin II-mediated vasoconstriction and aldosterone release with subsequent plasma volume expansion, resulting in hypertension; and (*2*) angiotensin II plays an important role in the progression of renal diseases. In fact, angiotensin II effects on hemodynamics, such as in-

creased systemic and glomerular blood pressure, and on tissue growth, including promotion of mesangial hypertrophy and fibrosis, are both thought to be responsible for progressive loss of renal function [25, 26]. Even more relevant in this regard could be the recent description of the intrarenal RAS [27, 28], which is thought to contribute to long-term blood pressure regulation by integrating distant tubular sodium-reabsorbing functions.

To minimize the probability of equivocal results, a sufficiently large sample size (260 ESRD patients and 327 controls) with a high ethnic homogeneity and an accurate definition of the renal phenotype was selected. Identified polymorphic allele frequencies for the *AGT* and *CYP11B2* genes were in accord with frequencies reported for the general white population [6, 18]. The *ACE-D* allele frequency was 47%, slightly lower than the reported range of 50 to 63% for Caucasians [7, 8, 11]. This was expected, however, since we used repeated PCR with intron-specific primers using single-step PCR to avoid overestimation of the *DD* frequency [29].

There was a genotype balance between control subjects and ESRD patients for the *AGT*, *ACE*, or *CYP11B2* genotypes. This observation indicates that polymorphisms in the RAS genes investigated do not account for the occurrence of renal diseases per se. The most interesting finding was the association of the *AGT-MM* genotype with the absence of nephropathy and of the *AGT-TT* genotype with ESRD in patients with diabetes mellitus (Fig. 1). An association of *AGT-T* with susceptibility to nephropathy in diabetes mellitus type 1 in a mixed population from Boston [30] or type 2 in aboriginal Canadians [31] has been previously reported. However, no association was found between the *AGT* genotype and nephropathy [32] or microalbuminuria [33] in two distinct European populations with diabetes mellitus type 2, although a recent study in insulin-dependent diabetics showed an association of the T allele of *AGT* with elevated urinary albumin excretion [34]. These contrasting findings might be due to either ethnic variance or gender differences, since the association of the *AGT* genotype with diabetic nephropathy has been found in men but not women [30]. To reconcile the contrasting findings and to confirm our present data, a prospective study with a larger number of patients with diabetes mellitus, in which the onset of renal disease from microalbuminuria to clinical nephropathy to end-stage renal failure will be monitored in relationship to the *AGT* genotype, ethnic background and gender are necessary.

The *ACE-DD* homozygote renal patients showed a more rapid progression to ESRD than *ACE-DI* or *II* subjects (Fig. 2A). The risk associated with the *ACE-D* allele has been found to be more apparent in renal patients without proteinuria or hypertension [35]. Moreover, the *D* allele of the *ACE* gene was associated with increased urinary albumin excretion in essential hypertension [36]. In our population, however, the effect of *ACE* gene *I/D* polymorphism on the rate of progression to renal failure, analyzed by the presence or absence of proteinuria or hypertension, did not show any significant differences. This might be due to the small number of patients in each subgroup. A significant correlation between the rate of progression of renal failure and the *AGT* genotype was found for patients with glomerulonephritis of any etiology (Fig. 3), an observation in accordance with the report of Pei et al of a more rapid progression with the *AGT-TT* than with *AGT-TM* or *AGT-MM* alleles in IgA nephropathy [37]. The observed effects of the *ACE* polymorphism in all renal patients and *AGT* polymorphism in patients with glomerulonephritis were not biased by differences at the time the renal disease was determined, as our patients were comparable for renal function and incidence of proteinuria or diabetes mellitus at the time of renal diagnosis among the different genotype subgroups. No association was found between *AGT*, *ACE*, and *CYP11B2* gene polymorphism and the age of onset of ESRD, the etiology of renal disease, the presence of hypertension, and the number of antihypertensive drugs used to control blood pressure.

Whenever an association between a gene polymorphism and a phenotype is found, two possibilities need to be considered. First, the observed association may be due to the effect of another gene mapping in close proximity to the one under study. Second, the interactive effect of two genes mapping on different chromosomes and both with a plausible role in causing the phenotype may lead to an underestimation or an overestimation of the involvement of the gene under study in determining the phenotype. This latter aspect is of particular interest when the complexity of blood pressure regulation is considered [38]. The context dependency of a gene on the background of another gene in determining one phenotype has been observed in experimental models of hypertension [39] and in humans [40, 41]. When interactions between the three genes were analyzed, no significant difference was found between controls and ESRD patients for the *AGT-ACE*, *AGT-CYP11B2*, or *ACE-CYP11B2* genotypes. However, the *ACE-DD* genotype was found to predict rapid progression to ESRD in *AGT-MM* homozygotes (Fig. 2B). Interestingly, an interaction between *AGT* and *ACE* polymorphisms in IgA nephropathy has already been reported [37], with rapid progression being associated with the *AGT-TT* allele regardless of the *ACE* genotype, while *AGT-MM* homozygotes had a more rapid progression only when the *ACE-DD* genotype was present.

These findings indicate that *ACE* gene polymorphism is a predictor, albeit weak, of progressive renal function loss in patients with renal disease. A relatively stronger influence of *ACE* genotype seems apparent in patients with glomerulonephritis who are homozygous for the

AGT-MM genotype. The latter has been associated with lower AGT plasma levels as compared with the *AGT-TT* genotype [6]. The functional significance of the *ACE* gene *I/D* polymorphism has been the object of debate. Plasma ACE activity is higher in *DD* homozygotes than in II homozygotes [7], and pressure response to exogenous angiotensin I is increased in *DD* homozygotes [42]. However, plasma levels of RAS components, that is, renin, angiotensin II, and aldosterone, were reported similar in various *ACE* genotypes [43]. It is possible that the intrarenal RAS may play a pivotal role with regard to the interaction between *ACE* genotype and progression of renal diseases independently of plasma renin and angiotensin levels [27, 28]. In fact, an exaggerated renal vasodilator response to angiotensin blockade was observed in diabetic nephropathy despite low plasma renin [27]. The role of the *ACE* gene *I/D* polymorphism in renal disease has been recently reviewed by Navis et al [44]. They identified 16 mostly retrospective studies addressing the issue of *ACE I/D* polymorphism and progression of renal function loss. In most studies, the sample size was rather limited (10 studies, $N < 200$). In the presence of the *D* allele there was an increased risk of long-term renal function loss, particularly in patients with both insulin-dependent and non–insulin-dependent diabetes mellitus. However, studies in patients with IgA nephropathy, autosomal dominant polycystic kidney disease, and renal conditions of diverse origin yielded mixed results [44]. These differences in the body of data can be explained by several factors. The interpretation of data is certainly complicated by methodological limitations inherent to studies of multifactorial diseases by association analyses. Inadequacy in the sample size, genetic heterogeneity, and inaccurate definition of the renal disease phenotype are additional limiting factors. We have attempted to minimize these pitfalls by selecting a sufficiently large number of patients with ESRD from one single center and of control subjects. Moreover, our population can be considered fairly homogeneous, with more then 88% of the subjects originating from the Swiss German area. Renal disease phenotype was also defined strictly, with only 16% of patients suffering from a renal condition that was not clearly classifiable. Nevertheless, we cannot exclude a selection bias inherent to retrospective studies. For instance, the *ACE* genotype appears to determine cardiovascular mortality [45], and if this was also the case in our population with renal diseases, an increased mortality before ESRD is reached would lead to an underestimation of alleles promoting renal function loss. Recent data also suggest a possible effect of the *ACE-DD* on the response to ACE inhibitor therapy [24, 46, 47]. Thus, ACE inhibitor therapy could represent a possible confounding factor in the present investigation. However, in a previous report, the *DD* genotype was associated with a resistance to ACE inhibitors [46], while another study suggested that progression to ESRD may be more effectively reduced in patients with the *DD* genotype [24, 47]. In the present population, progression to ESRD was comparable among men and women with the *DD* genotype but was shorter in men with the II or *ID* genotype, an observation in line with a previous report [24], thus suggesting a limited confounding effect of ACE inhibitor therapy.

The functional relevance of the *M235T* polymorphism of the *AGT* gene and the *−344C/T* polymorphism of the *CYP11B2* is a topic under investigation by several groups. A study in selected hypertensive subjects in two distinct American and French populations demonstrated a higher frequency of the *M235T* variant of the *AGT* gene in hypertensive patients than in controls [6]. Studies in white Europeans and African Caribbeans on the other hand failed to demonstrate a significant linkage with *AGT M235T* variants and hypertension [48, 49]. The same is true for the polymorphism in the promoter region of the *CYP11B2* gene at position −344 of the SF1 binding site, where some authors described a weak association with hypertension [18, 19, 21]. In the present population, there was no association between the *CYP11B2* genotype and either hypertension in the patients without renal failure or rate of progression of renal disease in the ESRD population. Therefore, the *−344C/T CYP11B2* genotype is probably not a primary candidate for susceptibility or progression of renal disease.

In conclusion, susceptibility for ESRD and faster progression to ESRD are linked with the *AGT* genotype in diabetic patients. Faster progression to ESRD is associated with the *ACE* genotype when the total population with ESRD and with the *AGT* genotype when patients with glomerulonephritis are considered. Thus, genes of the RAS system are candidate genes for the understanding of the interindividual differences in the development and course of ESRD.

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